

Fourier Transform and Fourier Optics EE5136

Final Project (Spring 2025): Super-resolution Microscopy

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Abstract: This is the Final Project for the course "Fourier Transform and Fourier Optics" at National Taiwan University. In the term report, I will go through two specific technology improvement of Microscopy, where the concept of super-resolution has overcome the physical diffraction limit and provide even higher resolution than airy disk. With the explanation through Fourier Optics and Fourier Transform, we can discover how Structured illumination Microscopy and pixel reassignment achieve twice the resolution of traditional wide-field Microscopy and attains super-resolution. Since SIM is of low phototoxicity and high field of view, it plays an important role in live-cell imaging and long-term imaging nowadays. Pixel reassignment as well is widely adopted in Microscopy to attain better resolution without huge modifications to the overall system structure.

1. Introduction

Microscopy is a long-investigated technology where lots of efforts have been made to further improve the resolution to observe even smaller structure. According to Ernst Karl Abbe's research, the diffraction of light will limit the resolution of traditional microscopy, especially for wide-field microscopy. With non-ideal point spread function for an optical system, object lens of microscope for example, the imaging will be blurred. Thus, with pure optical system, the resolution can't be infinitely high or even break the abbe law. With intensive devotion and researches, the Nobel Prize in Chemistry was awarded to Eric Betzig, W.E. Moerner and Stefan Hell for their dedication to the development of super-resolved fluorescence microscopy, which brings optical microscopy into nanometers' scale. Since then, super-resolution microscopy has become an important research topic for the enhancement of microscopy.

2. Super-resolution Microscopy

2.1. Microscopy

Traditional Microscopy techniques involve objective lens, eyepiece, or dichroic mirror (confocal microscopy) placed with distance of 2-pi or 4-pi, where Fourier Optics play an significant role in the optical design. This is due to the reason of the correspondence between spatial domain and frequency domain. Lenses act as a Fourier Transform element, where the imaging can be explained by Fourier Optics. Filtering or de-convolution can be done in frequency domain, for example, to improve the system performance after transforming back to the spatial domain. This forms the backbone and main idea of the modern microscope.

To practically validate that with pure optical system design optimization is not sufficiently capable of resolving beyond diffraction limit, I simulate a microscope objective from a google patent [2] prototype and optimize it. From my ZEMAX simulation result in Fig. 1, we can discover that even after thorough optimization with 7 lenses, the point spread function still can't overcome the diffraction limit, where Fig. 1(b) clearly shows the results. (Airy disk is marked in black solid line.)

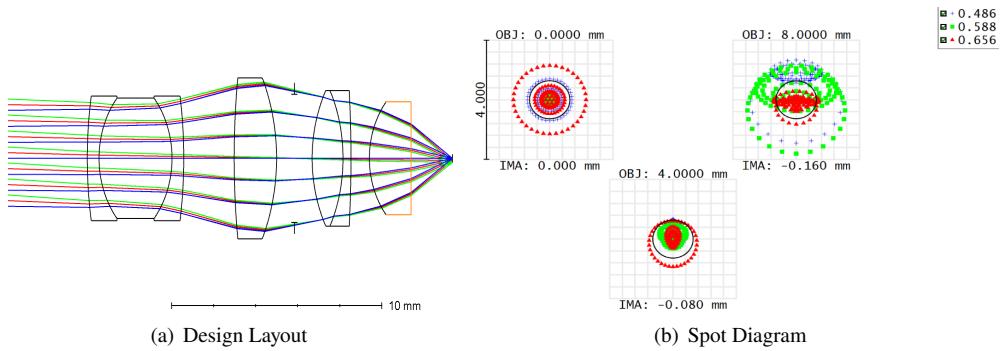


Fig. 1. Microscope Objective optimized using ZEMAX from patent, still fails to break the limitation of diffraction limit (reference: [2])

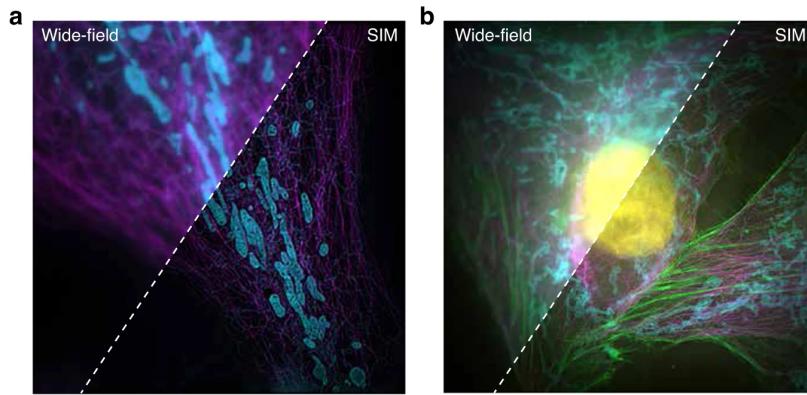


Fig. 2. Super-resolution SIM that improves resolution (Reference: [4])

2.2. Super-resolution

As shown in the simulation, traditional microscope can't resolve things that are smaller than or equal to its airy disk. This is where super-resolution microscopy comes into play. There are lots of super-resolution microscopy techniques, such as STED, SIM, and STORM. Some of them take advantages of chemical or medical properties to achieve super-resolution. For instance, [3] STED Microscopy uses excitation beam and depletion beam to form a donut-shape to suppress part of components and attain super-resolution.

Aside from these techniques, Structured Illumination Microscopy (SIM) projects high frequency structured light onto the objects. This will form the Moire pattern, which can be further processed and reconstructed back with more high frequency information that are originally being lost (outside the cutoff frequency of the optical transfer function of the optical system); thus, attain super-resolution.

From Fig. 2, it's clearly seen that with SIM, the resolution can be improved significantly. It can then be utilized to see finer details that are not observable with wide-field microscope.

3. Structured Illumination Microscopy

In this section I will explain how Structure Illumination Microscopy is implemented in the aspect of Fourier Optics, where Fourier Transform is of utmost importance to the core concept of SIM.

3.1. Notations

Table 1. Notations for the explanation in this section

Notations	Meanings	Notations	Meanings
$i_{ill}(\vec{r})$	Illumination Intensity (in cosine)	$o(\vec{r})$	Fluorophore Distribution
$PSF(\vec{r})$	Point Spread Function	k_c	Cut-off Frequency
$OTF(\vec{f})$	Optical Transfer Function	k_p	Pattern Spatial Frequency
ϕ_p	Phase of Pattern	$g(\vec{r})$	Final Image

3.2. Mathematical Backgrounds

First, we describe the structured illumination intensity in cosine wave that is repeated in pattern. (m is modulation depth, $0 \leq m \leq 1$)

$$i_{ill}(\vec{r}) = i_{ill}(x, y) = i_0[1 + m \cdot \cos(\vec{k}_p \cdot \vec{x} + \phi_p)] \quad (1)$$

This is accomplished by illuminating the sample with a spatially structured pattern, a sinusoidal grid. The interaction between this illumination pattern and the sample's structure creates Moiré fringes, which are a key element of this technique. The resulting fluorescence emission from the sample is, thus, the product of the sample's structure and the illumination pattern. That is, it can be ideally represented as $o(\vec{r}) \cdot i_{ill}(\vec{r})$. However, we know that an optical system can't be perfectly ideal, the actual resulting image should be further convolved with the system point spread function (Consider 2D-convolution here).

$$g(\vec{r}) = [o(\vec{r}) \cdot i_{ill}(\vec{r})] * * PSF(\vec{r}) \quad (2)$$

To further understand the improvement in resolution, we should resort to the frequency domain with Fourier Transform. That is to say, we manipulate Eq. (2) to get its Fourier Transform equation. Note that the Fourier transform of the Point Spread Function is the Optical Transfer Function, which acts as a low-pass filter, defining the cutoff frequency of the microscope. Any spatial frequencies in the object beyond this cutoff are lost. The Fourier Transform of the final image $g(\vec{r})$ is $G(\vec{k})$, which can be equated as:

$$g(\vec{r}) = [o(\vec{r}) \cdot i_{ill}(\vec{r})] * * PSF(\vec{r}) \supset \mathcal{F}\{o(\vec{r}) \cdot i_{ill}(\vec{r})\} \cdot OTF(\vec{k}) = G(\vec{k}) \quad (3)$$

where

$$\mathcal{F}\{o(\vec{r}) \cdot i_{ill}(\vec{r})\} = O(\vec{k}) * * I_{ill}(\vec{k}) \quad (4)$$

and

$$o(\vec{r}) \supset O(\vec{k}) ; i_{ill}(\vec{r}) \supset I_{ill}(\vec{k}) \quad (5)$$

are the Fourier Transform pairs. We can now first expand and calculate the Fourier Transform of illumination Intensity pattern:

$$I_{ill}(\vec{k}) = I_{ill}(k_x, k_y) = i_0[\delta(k_x, k_y) + \frac{m}{2}\delta(k_x - k_p, k_y) + \frac{m}{2}\delta(k_x + k_p, k_y)] \quad (6)$$

We can clearly see that there are three delta functions in the expression. We can later see that this spectrum characteristic is significant to the improvement of super-resolution SIM.

Now, back to spatial domain. When we multiply the object by the structured illumination, we get:

$$\begin{aligned}
o(\vec{r}) \cdot i_{ill}(\vec{r}) &= o(x, y) \cdot i_0 [1 + m \cdot \cos(\vec{k}_p \cdot x + \phi_p)] \\
&= i_0 \cdot o(x, y) + \frac{i_0 \cdot m}{2} \cdot o(x, y) e^{j(k_p x + \phi_p)} + \frac{i_0 \cdot m}{2} \cdot o(x, y) e^{-j(k_p x + \phi_p)} \\
&\supset i_0 \cdot O(kx, ky) + \frac{i_0 \cdot m}{2} e^{j\phi_p} \cdot O(kx - k_p, ky) + \frac{i_0 \cdot m}{2} e^{-j\phi_p} \cdot O(kx + k_p, ky)
\end{aligned} \tag{7}$$

This is a crucial result, which can be simply understood by comparing with Eq. (6). Product in spatial domain corresponds to 2D convolution in frequency domain. And that $O(kx, ky)$ convolves with $I_{ill}(\vec{k})$ is the replication of $O(kx, ky)$ in three different frequencies with different amplitudes. That is, the object's Fourier spectrum appears at three different locations in frequency space: First, centered at the origin, which is the DC component. Second, shifted by $+k_p$, which is the positive first-order component. And lastly, shifted by $-k_p$, which is the negative first-order component.

3.3. Resolution Improvement

With the aforementioned derivation of the spectrum, we now have Eq. (7). Since we can choose the grating frequency k_p to be close to cutoff frequency k_c of the optical system, it's possible that high-frequency components of the object (normally beyond k_c) are shifted down by k_p such that they fall within the passband of the detection system. To be more specifically, object frequencies up to $(k_c + k_p)$ can now be detected because they appear at frequency $(k_c + k_p - k_p) = k_c$ after the shift.

For instance, if we choose $k_p = k_c$, the maximum detectable frequency becomes:

$$k_c + k_p = k_c + k_c = 2k_c \tag{8}$$

This corresponds to twice the resolution improvement, which is the ideal enhancement of the resolution using structured illumination microscopy. With this 2x resolution improvement, it's possible to achieve super-resolution. It can be discovered with reference to Fig. 1 if you consider twice the isotropic resolution improvement. In this case, the imaging can be all inside the airy disk, which is previously impossible to achieve with pure optical manipulation.

In general, the total observable region in Fourier space becomes the union of three circles, which are often seen in the visualization of the structured illumination microscopy. That is, we denote the original passband as that $|k| \leq k_c$. Then, the two shifted regions are $|k - k_p| \leq k_c$ and $|k + k_p| \leq k_c$, which corresponds to positive shifting and negative shifting region respectively. With $k_p = k_c$, these three circles create a region that extends to $2 \cdot k_c$, doubling the resolution.

It's important to note that with single structured illumination light projecting onto the object as described in Eq. (1), the resolution improvement happens primarily in the direction of the repeated pattern anisotropically. In our case as Eq. (1), the improvement is mainly along the x direction.

3.4. Number of Frames

As shown above, a single frame with structured light is not sufficient for isotropic 2D SIM imaging. Each orientation of the grating requires multiple phase shifts to separate the three frequency components. For a single grating orientation, we need at least 3 different phases to solve for the three unknown components. To illustrate this, we can consider the system with three phases:

$$\phi_n = \frac{2\pi n}{3}, \text{ where } n = 0, 1, 2. \tag{9}$$

In this case, we get the system to be equated as:

$$\begin{aligned} I_1(x, y) &= A + B \cdot \cos(k_p \cdot x) + C \cdot \sin(k_p \cdot x) \\ I_2(x, y) &= A + B \cdot \cos(k_p \cdot x + \frac{2\pi}{3}) + C \cdot \sin(k_p \cdot x + \frac{2\pi}{3}) \\ I_3(x, y) &= A + B \cdot \cos(k_p \cdot x + \frac{4\pi}{3}) + C \cdot \sin(k_p \cdot x + \frac{4\pi}{3}) \end{aligned} \quad (10)$$

where A : DC component ; B, C : The shifted components

This 3×3 system can be solved to extract A , B , and C independently. Besides, to achieve isotropic resolution improvement, we need gratings in multiple orientations. The minimum number for 2D SIM is typically 3 orientations: $0^\circ, 120^\circ, 240^\circ$, for example. As a result, each orientation requires 3 phase-shifted images to separate the frequency components, and we need 3 orientations for isotropic coverage. Therefore, total frames needed are $3 \text{ orientations} \times 3 \text{ phases} = 9 \text{ frames}$.

3.5. Reconstruction

Structured Illumination Microscopy utilizes structured light shined on object and collects the resulting images. It requires parameters (some methods use fixed parameters calculated in advance and some are adaptive, PCA-SIM [5] for example) to reconstruct these information back to an enhanced final image. This post-processing involves techniques like wiener filter, Richardson–Lucy deconvolution, and so forth. Though these intensive processing aim at improving the quality and smooth the images, artifacts induced by the pipeline are often the limitation for the quality of SIM. Artifacts like sidelobe, honeycomb often make practical SIM from achieving ideal improvement of twice the resolution.

The reconstruction process generally includes four steps: (1). Separate Frequency Components, (2). Shift Components Back, (3). Combine All Orientations, and (4). Inverse Fourier Transform. Firstly, we need to solve the linear system to extract the DC and shifted components for each orientation. From Eq. (10) with more general representations, we have:

$$\begin{aligned} [A(kx, ky)] &= [1 \quad 1 \quad 1][I_1(kx, ky)] \\ [B(kx, ky)] &= [1 \quad e^{j\phi_1} \quad e^{-j\phi_1}][I_2(kx, ky)] \\ [C(kx, ky)] &= [1 \quad e^{j\phi_2} \quad e^{-j\phi_2}][I_3(kx, ky)] \end{aligned} \quad (11)$$

Then, we have to move the shifted frequency components back to their correct positions. For example, we will shift $B(kx, ky)$ by $-k_p$ and shift $C(kx, ky)$ by $+k_p$ in this case. After this, we will combine the shifted components from all three orientations in Fourier space, and taking extra care to properly weight overlapping regions. Lastly, it's needed to apply the inverse Fourier Transform to obtain the super-resolved image eventually.

3.6. Mathematical Constraints and Limitations

As detected by sensors, Nyquist Sampling plays an important role. The grating period must satisfy that it's $\geq 2 \times (\text{resolution limit of detection system})$. In addition, Signal-to-Noise also poses certain limitation on the method. We know that the reconstruction process involves dividing by the Optical Transfer Function, which amplifies noise at high frequencies. This places demanding requirements on the signal-to-noise ratio of the input images.

4. Pixel Reassignment

With similar physical concept to how Structured Illumination Microscopy achieves super-resolution, pixel reassignment on Image Scanning Microscopy (ISM) is a technique that shifts

high frequency information that are originally lost back to the region inside the system's Optical Transfer Function. Different from SIM which utilizes different phases and orientations to accomplish this (the incident angle represents the frequency in the aspect of Fourier Optics), pixel reassignment reassign the relative position of collected information and improve the resolution to ideally twice the original result. An important note is that ISM is a scanning microscopy

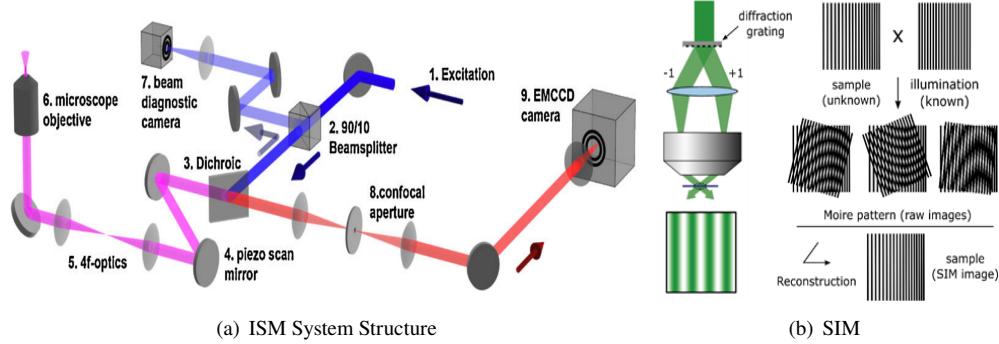


Fig. 3. System Structure of ISM and SIM (reference: [7])

originated from confocal microscopy that utilizes pinhole aperture to restrict the light to the size of a point on the object as depicted in 7, 8, and 9 in Fig. 3(a). On the other hand, SIM is more like a Fluorescent Microscope that shine on a larger range on object but different from the illumination pattern, where SIM uses a sinusoidal illumination pattern instead of uniform illumination used in Fluorescent Microscope.

4.1. Notations

Table 2. Notations for the explanation in this section

Notations	Meanings
n	Refractive index of immersion medium
α	Semi-angular aperture of the microscope objective
v_{dmax}	Circular detector array of normalized radius
x_s	Scan Position
x_d	Pixel Position on detector
$F(\cdot)$	Fluorescent distribution on sample
$H_1(H_2)$	Illumination (Detection) Point Spread Function
$C_1(C_2)$	Excitation (Emission) Transfer Function

4.2. Mathematical Backgrounds

We should first understand the operation of ISM, where signals are recorded from a point object at different points in the detector plane as the sample is scanned. We adopt two coordinates to describe the system, one is the detector element position, $v_d = \frac{2\pi}{\lambda}x_d \sin(\alpha_d)$, and the other is

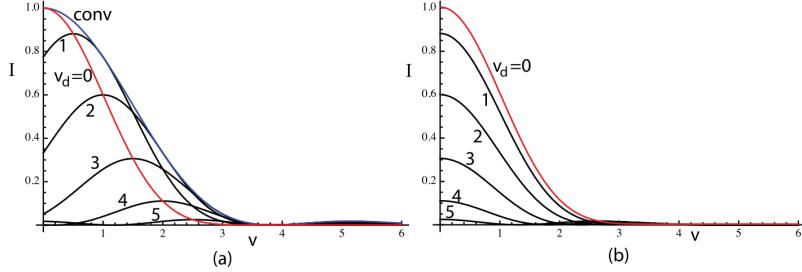


Fig. 4. Pixel Reassignment Operation (Reference: [6])

normalized transverse coordinate of the scan position, $v = \frac{2\pi}{\lambda} n x s \sin(\alpha)$. That is, we can take $v_d = 0$ as corresponding to a point on the axis, a confocal image result.

We know that as v_d increase: peak of image intensity decrease, peak shifts sideways, image is sharper, and no longer circular symmetric. If we integrate these signals over the complete plane, it will give rise to non-confocal image that is equivalent to a conventional image. This is definitely not a desired result. The idea to tackle this is that: if the images from the off-axis detector points are shifted, they add up to give a sharper point spread function (PSF). Fig. 4 gives a visualization of how pixel reassignment is operated.

We start from considering the signal recorded at a point x_d, y_d in the plane of the detector for a two-dimensional fluorescent object of strength $F(x' - x_s)$ at scan position x_s is:

$$I(\mathbf{x}_s, \mathbf{x}_d) = \iint H_1(\mathbf{x}') F(\mathbf{x}' - \mathbf{x}_s) H_2(\mathbf{x}' - \mathbf{x}_d) d^2 \mathbf{x}' \quad (12)$$

By substitution:

$$\mathbf{x}_s = -\mathbf{x}_1, \mathbf{x}_d = \mathbf{x}_2 - \mathbf{x}_1, \mathbf{x}' = \mathbf{x} - \mathbf{x}_1 \quad (13)$$

which is essentially corresponded to scanning the illumination beam instead of the specimen. This substitution change the viewpoint from specimen side to the progression of beam. Eq. (12) can be first changed in to Eq. (14) and then be further written in symmetrical form for the four-dimensional signal.

$$I(\mathbf{x}_1, \mathbf{x}_2) = \iint H_1(\mathbf{x} - \mathbf{x}_1) H_2(\mathbf{x} - \mathbf{x}_2) F(\mathbf{x}) d^2 \mathbf{x} \quad (14)$$

The signal from measurement x_d reassigned to the point $\mathbf{x}_r = (1 - a)\mathbf{x}_1 + a\mathbf{x}_2$ and summed with pinhole weighting factor $S(\mathbf{x}_d)$ (1 inside a specific radius, 0 otherwise). We can get:

$$I(\mathbf{x}_1, \mathbf{x}_2) = \iiint \iint H_1[\mathbf{x} - \mathbf{x}_r - a\mathbf{x}_d] H_2[\mathbf{x} - \mathbf{x}_r + (1 - a)\mathbf{x}_d] \times F(\mathbf{x}) S(\mathbf{x}_d) d^2 \mathbf{x} d^2 \mathbf{x}_d \quad (15)$$

$\forall a \text{ with } 0 \leq a \leq 1$

This representation can be understood by dividing into three parts. When $a = 0$, the system becomes a scanning (non-confocal) microscope, equivalent to integration without reassignment.

As for $a = \frac{1}{2}$, this is just the pixel reassignment as described above; while for $a = 1$, the system reduces to a conventional microscope. The observation serves as a great hint on how a can affect the results.

However, the aforementioned derivations lie on spatial domain analysis. We should transform it to frequency domain to assess further on the properties of pixel reassignment.

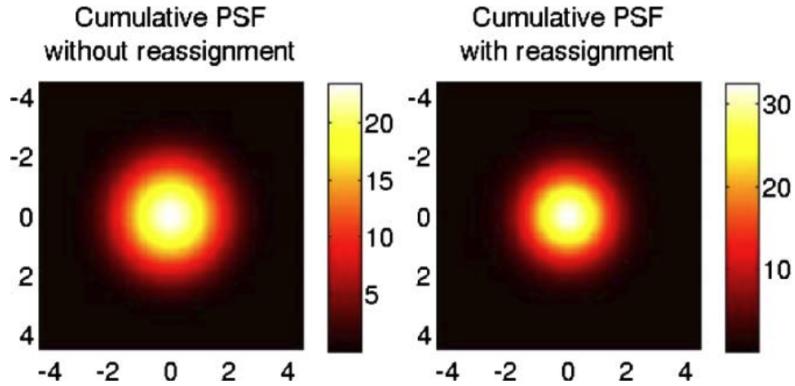


Fig. 5. Transverse PSF due to summation of effective PSFs without and with reassignment. (Reference: [6])

If $S(\mathbf{x}_d) = 1$, using a large detector, the integral in x_d gives the effective PSF as the 2D convolution of two scaled, 3D PSFs (illumination and detection PSFs). Thus, $C_{eff}(\mathbf{m})$ is the product of two scaled, illumination and detection, optical transfer functions (OTFs) in x , y , convolved in the axial direction. (where \mathbf{m} is in-plane spatial frequency.)

$$C_{eff}(\mathbf{m}) = C_1[(1-a)\mathbf{m}]C_2[a\mathbf{m}] \quad (16)$$

This result can be split into two parts: (1). In x , y directions we have product of two scaled OTF. (2). In z direction we get a 1D convolution. This comes to a significant results that the resolution in transverse directions can maximumly be doubled with the cutoff frequency being doubled ($a = \frac{1}{2}$). However, the resolution enhancement is not isotropic in three directions.

Consider that As $C_1(0) = C_2(0) = 1$, independent of the presence of defocus, $C_{eff}(0) = 1$, so that there is no optical sectioning in axial direction when pixel reassignment is used with a large detector array. This limits the applications of pixel reassignment in some cases, such as thick samples.

The effectiveness of pixel reassignment can be discovered in Fig. 5, where the transverse PSF is significantly shrunk after reassignment. We can observe this from the smaller radius and the increasing amplitude, which can also be understood from Fig. 4 after summation and proper normalization. However, note that in actual case, the twice resolution improvement is seldom achieved. Slightly worse performance takes place due to several practical issues and mechanical problems.

5. Code Simulation

As SIM reconstruction requires parameters estimation stage, which is for the acquirement of parameters that are further used in reconstruction stage. With pure simulation, there are some well-maintained MATLAB toolboxes that are available online (e.g. SIMToolbox). However, it requires several techniques to deal with possible artifacts. In this project, I intend to implement simple SIM that process images as what I've stated in this report. Though I've tried several methods and referred to lots of papers, the reconstruction results can't be as great as what theoretically predicts.

I implement a full pipeline to of SIM to simulate, reconstruct, and compare results under different conditions. I set the test image resolution as 512 x 512 which includes both high-frequency and medium-frequency sinusoidal line patterns. Additionally, four Gaussian "point"

sources are added to the test image.

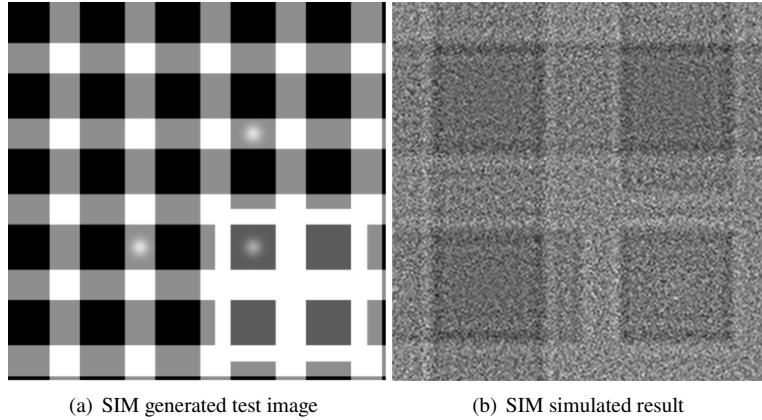


Fig. 6. Simulation on SIM to improve resolution under manually added Gaussian blur

As depicting in Fig. 6(a), the generated test image contains both high-frequency and low-frequency information. My reconstruction result is shown in Fig. 6(b), where it displays the result of the bottom-right of the input test image. Though not being the same to the test image, the reconstructed image captures several details with both high and low frequency patterns. The difference between SIM and Wide-Field Microscopy can prove the enhancement, where Fig. 7(b) highlights several information captured by SIM that are not fully obtained by Wide-Field Microscope.

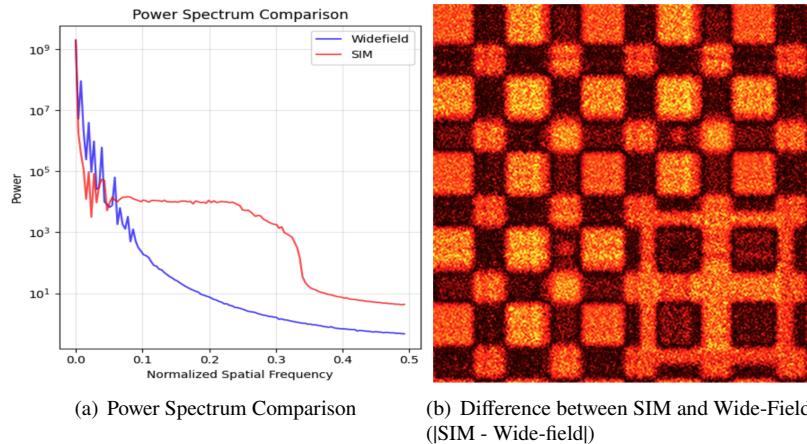


Fig. 7. Simulation on SIM to compare with wide-field microscopy with manually added Gaussian blur

We can also observe Fig. 7(a) to see how powers are distributed and how do they change with frequency. It's obvious that SIM reconstructs image with better quality and more high-frequency information included.

6. Conclusion

In this report, I mathematically explain how SIM (Structured Illumination Microscopy) and Pixel Reassignment achieve super-resolution using Fourier Optics and Fourier Transform. The core idea behind the two methods are basically shifting or reassigning the position in frequency domain to include more information in the cutoff frequency of OTF. With optimized optical system design of microscope objective lens in Fig. 1, we are able to appreciate the novelty and effectiveness of super-resolution microscopy. And with the simulation of SIM, we get to know the changes brought by such algorithms in practice.

These researches and implementations again showcase how Fourier Transform and Fourier Optics are of utmost importance to several fields. The ideas of Fourier Optics provides itself as a valuable tool for analyzing these phenomenon. Despite the micro-scale like microscopy, it can also extend to macro-scale like astronomy and telescope. All in all, I hope the term report conveys my understandings and researches in the topic of super-resolution microscopy thoroughly. With the advancement of the technology, I also look forward to how it can benefit human beings and unravel the unknown.

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